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CHEMOMODULATORY EFFECT OF DIOSGENIN ON XENOBIOTIC DETOXIFYING ENZYMES AND FREE RADICAL SCAVENGING ACTIVITIES

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ABSTRACT

Considerable attention has been paid to identify potential modulators of free radical scavengers. Diosgenin, a steroidal sapogenin is tested in Swiss albino mice with 50 mg/kg body weight and 30 mg/kg body weight, per oral route based on acute oral toxicity test. Effect on mice serum glutamate-pyruvate transaminase (SGPT) and serum glutamate-oxaloacetate transaminase (SGOT) levels were considered. Hepatic system of mice recorded significant elevation in the specific activity of Cytochrome P_{450} and Cytochrome b_5. Increase in specific activity of GST was evident with augmentation of GSH level. Diosgewnin positively regulated the specific activity of glutathione reductase (GR) and glutathione peroxidase (GPx). Catalase levels recorded an increase with a sharp decline in lipid peroxidation. Our study has established that diosgenin is bifunctional inducers of xenobiotic detoxifying enzymes and possess significant free radical scavenging potential, providing a lead for development novel antioxidant.

KEYWORDS: Chemomodulatory Effect, Diosgenin, Xenobiotic Detoxifying Enzyme, Free Radical

INTRODUCTION

Active oxygen species and free radicals play an important role in the pathogenesis of several human diseases like rheumatoid arthritis, cardiovascular diseases and cancer development (Hertog et al., 1997).

Liver is the principal organ responsible for drug metabolism and appears to be sensitive target site for substances modulating biotransformation (Gram and Gillete, 1971). Xenobiotics enzymes can be categorized in to Phase I and Phase II enzymes- Phase I enzymes (Cytochrome P_{450} and Cytochrome b_5) begin the biotransformation by oxidizing, reducing or hydrolyzing toxins, creating biotransformed intermediate and the Phase II enzymes perform conjugation reactions which help to convert these intermediates to less toxic water soluble substances that easily excreted or eliminated from the body (Percival, 1997). Glutathione S-transferase (GST) forms an important component of phase II metabolism and it play a physiological role in initiating the detoxification of many alkylating agents (Leaver and George 1998).The effect of free radicals can be mitigated by cellular scavengers such as reduced glutathione (GSH), glutathione reductase (GR), glutathione peroxidise (GPx), superoxide dismutase (SOD) and catalase (CAT) (Froni and Wilson, 1983).Therefore, a study regarding regulators of xenobiotic detoxifying enzymes and free radical scavengers can provide an understanding about the disease preventive prospective. Diosgenin, also known as (25R)-Spirost-5-en-3-beta-ol, 22alpha-Spirost-5-en-3beta-ol, has been shown to possess wide range of activities. It is used as a dietary supplement, in combination with interferon-α, exerted an additive effect on the 44444 resultant anti-HCV activity (Wang et al., 2011). *In vitro* effect of diosgenin, isolated from *Asparagus officinalis* was studied in a dopamine model of oxidative stress in isolated rat synaptosomes (Kondeva-Burdina et al., 2007). Diosgenin has shown to be useful in the maintenance of healthy blood cholesterol levels and is the primary material for the synthesis of number of hormonal products such as 5-Dehydroepiandrosterone (5-DHEA), a 19-carbon endogenous natural steroid hormone in humans (Derrida, 2003).

MATERIALS AND METHODS

Test Material

Diosgenin [IUPAC name: (3β, 25R)-spirost-5-en-3-ol; $C_{27}H_{42}O_{3,}$ (414.62 g mol^{-1}), E.C. 208-134-3] was purchased from Sigma-Aldrich Co., St. Louise, USA (lot no.079K1096).

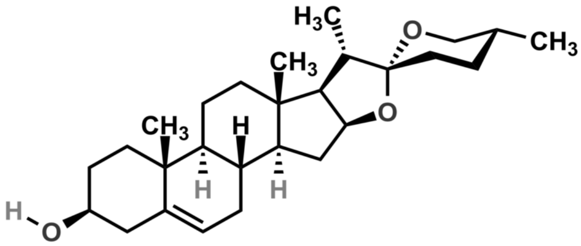

Figure 1: Chemical Structure of Diosgenin [(3β, 25*R*)-Spirost-5-en-3-ol]

Test Animal

Randomly bred Swiss albino mice were *(Mus musclus)* maintained in the animal house, Department of Biotechnology, Gauhati University, Guwahati and sacrificed as per the guidelines of Animal Ethical Committee of Gauhati University (Regd. No. 902/AC/05/CPCSEA). Both male and female mice of 6-8 weeks of age weighing about 20± 2g were used.

Oral route of administration of the test material was selected as it is the preferred method of practical preventive measures and provides better absorption and physiological distribution of the test agent (Singh et al., 2002).

Acute Oral Toxicity Test and Dose Selection

Mice were randomly segregated into seven groups of 6 mice each; one group was maintained as negative control. Each group of animals were given graded dose of diosgenin. Behavioural changes, locomotion, convulsions and mortality were observed as toxicological parameters for 72 hours.

Based on the findings of acute oral toxicity test, a dose of 50mg/kg b. w. p. o. for diosgenin was used. Lower dose of 30mg/kg b. w. p. o. for diosgenin was tested in identical situation for comparing dose dependent effect of the modulator. Two groups, each comprising of 6 mice (both sexes), were treated with these two selected doses of modulators separately. The animals were observed for 14 consecutive days for any significant loss of body weight, behavioral changes, locomotory changes, convulsion and mortality. Another group of 6 mice was maintained as negative control.

Effect on Liver Function Parameters

To assure further safety of the dose selected, glutamate-pyruvate transaminase (GPT) and glutamate-oxaloacetate transaminase (GOT) were estimated by the method of Reitman and Frankel (1957). The liver somatic index, which is the ratio of liver weight to final body weight, was also calculated.

Effect on Xenobiotic Detoxifying Enzymes and Free Radical Scavenging System

Experimental Design

The animals were randomly assorted into the following four groups of six mice each:

Group I (n = 6): Animals received a normal diet and normal saline daily for 14 days and served as negative control

Group II (n = 6): Animals received a normal diet 30 mg/ kg body weight of diosgenin 14 days.

Group III (n = 6): Animals received a normal diet and 50 mg/ kg body weight of diosgenin 14 days.

Group IV (n = 6): Animals received a normal diet containing 0.75% BHA (Butylated hydroxyanisole) for 14 days and served as positive control.

Preparation of Homogenates, Cytosol and Microsomal Fractions

The animals were sacrificed and the entire liver was perfused immediately with 0.9% NaCl solution. The acid microsomal fractions were prepared by the method of Fry and Bridges (1975). The cytosol fractions were used for the assay of glutathione-S-transferase along with free radical scavenging system.

Assay Methods

Cytochrome P_{450} and Cytochrome b_5 content were assayed by the method of Omura and Sato (1964). GST activity was determined spectrophotometrically (Shimadzu UV180) at 37^0C as outlined by Habig et al., 1974. GSH was estimated as total non-protein sulphydryl group by the method as described by Moron et al., 1979. GR was assayed by the procedure of Carlberg and Mannervik (1985). GPx activity was measured by coupled assay method as described by Paglia and Valentine (1967).

SOD was determined by the method of Marklund and Marklund (1974) where as catalase activity was recorded as per the method described by Aebi (1984). Lipid peroxidation was evaluated by thiobarbituric acid reactive substances (TBARS) method given by Varshney and Kale (1990).

Statistical Analysis

Results are expressed as mean $\pm$ S.D. of 6 animals. Differences within the groups were analyzed by unpaired Student’s t-Test and one way ANOVA using Graph Pad Prism ver. 5.03, Graph Pad Software, San Diego, California, USA. Statistical significance was considered at $p < 0.05$ and $p< 0.01$.

RESULTS AND DISSCUSSIONS

Acute Oral Toxicity Test and Dose Selection

The highest (most concentrated) dose tested of diosgenin had not induced mortality in mice, hence, the LD_{50} of diosgenin could not be achieved. Higher concentration than tested dose could not be administered because of greater density of modulators in vehicle. There were no adverse effects (mortality and any apparent toxicity) on the animals at these given dose levels (30mg and 50mg/kg body weight/day for 14 days each).

These observations revealed that the selected doses are lower than the LD_{50}, which attribute to its more safety index. Identical procedure was adopted to study acute toxicity of extract of *Aphanamyxis polystachya* in male albino rat (Rao and Mishra, 1997). A previous study has determined the LD_{50} of diosgenin in mouse through oral route is higher than 8000mg/ kg body weight (www.mdidea.com/products/phytochemical/diosgenin).

Effect on Body Weight and Liver Function Parameters

The modulators did not affect the normal body weight of the test animals during the experimental period. The liver somatic index remained comparable to the control group indicating positive regulations of the modulator. Lack of any significant alteration in the GOT and GPT levels (Table 1) in the modulator treated groups in comparison to the control suggested safety aspects of the modulator at selected dose levels. Literature search has revealed that a range of diosgenin doses from 100 mg to 2000 mg/ Kg body weight of mice per day is considered safe (Mirunalini et al., 2011).

Table 1: Effect of Diosgenin on Body Weight, Liver Somatic Index and Liver Function Parameters

Group	Treatment(14 days)	Body Weight (g)		Liver Somatic Index	GOT (Unit/Liter)	GPT (Unit/Liter)
		Initial	Final			
I(n =6)	Vehicle control	20±1.23	21.4±2.29	5.43±0.21	72.33 ±4.11	75.44 ± 9.81
II(n = 6)	Diosgenin (30mg/kg b. w.)	20.7±3.34	21.1±2.17	5.59±0.10	71.93 ±5.70^{b}	75.04 ± 6.66^{a}
III(n = 6)	Diosgenin (50mg/kg b. w.)	20.1±0.71	20.9±1.81	5.71±0.21	72.39 ±4.09^{b}	75.19 ± 11.2^{a}

Values are expressed as mean ± S.D of 6 animals. 'a' and 'b' represent significant changes against negative control at $p< 0.01$ and $p < 0.05$ respectively.

Effect on Xenobiotic Detoxifying Enzymes and Lipid Peroxidation

Activities of Cytochrome P_{450} and Cytochrome b_5 in albino mice were enhanced by various folds when treated with both high and low doses of diosgenin for 14 days each. Microsomal Cyt P_{450} is a major electron transport chain of endoplasmic reticulum which plays a role in oxidative activation or inactivation of xenobiotics and promotes their excretion from the body by modulating the duration and concentration of their toxicity (Miller, 1988).

Table 2: Effect of Diosgenin and BHA on Xenobiotic Detoxifying Enzymes and Lipid Peroxidation

Group	Treatment (14 Days)	Cyt b_5 Mole/mg Protein)	Cyt P_{450} (n Mole/mg Protein)	GST (µ Moles of GSH-CDNB Conjugate Formed/min/mg Protein)	LPO (Malondialdehyde Formed/Mg Protein)
I(n = 6)	Negative Control	2.58 ± 0.04	0.21± 0.02	3.15 ±0.23	1.19± 0.2
II(n = 6)	Diosgenin(30mg/ kg b. w.)	2.64 ± 0.05^{a}	0.29 ± 0.07^{a}	3.60 ±0.02^{b}	0.65± .01^{a}
III(n = 6)	Diosgenin(50mg/ kg b. w.)	3.19 ± 0.11^{a}	0.41 ± 0.05^{a}	3.96 ±0.05^{b}	0.36± .01^{a}
IV(n = 6)	Positive Control (Normal diet containing 0.75% BHA)	3.82 ± 0.12^{a}	0.64+0.04^{a}	7.04 ±0.07^{b}	0.27± .01^{a}

Values are expressed as mean ± S.D of 6 animals. 'a' and 'b' represent significant changes at $p < 0.01$ and $p < 0.05$ respectively. BHA- Butylated hydroxyanisole; Cyt P_{450} – Cytochrome P_{450}; Cyt b_5 – Cytochrome b_5; GST – Glutathione S-transferase; LPO – Lipid peroxidation.

The hepatic GST activity was found to increase by diosgenin significantly in a dose dependent manner. The modulator is suggested to act as "blocking agent" and favours the sequential reduction of xenobiotic substrates preparing it for Phase-II metabolism. Ozen and Korkmaz (2003) also found similar increase in hepatic cytochrome b_5, glutathione S-transferase level in mice treated with *Urtica dioica* L. leaf extract. Table 2 also depict a significant inhibition in the lipid peroxidation (LPO) measured as the formation of malondialdehyde (MDA) production along with BHA treated groups. Any natural or synthetic agent with a capacity to lower the lipid peroxidation level can exert protection against oxidative stress. The level of MDA, an indicator lipid peroxidation, found to decrease significantly in diabetic test mice when experimented with *T. glaucescens* extract (Guy et al., 2008).

Effect on Free Radical Scavenging

As indicated in Table 3, a significant elevation of reduced glutathione was recorded as acid soluble sulphydryl (SH) group when treated with low and high doses of diosgenin for 14days. The BHA treated group also recorded an increase as expected. The elevated level of GSH protects cellular proteins against oxidation through glutathione redox cycle and also directly detoxifies reactive species (Ketterer, 1998). Both the doses of modulator had raised the activity of

GR as well as GPx. The increase in glutathione reductase level helps in maintaining the basal level of cellular GSH (Lopez-Baria et al., 1990). Diosgenin treatment showed a moderate augment in specific activity of catalase but was less than that of BHA treated animals. It has been proposed that glutathione peroxidase is responsible for the detoxification of hydrogen peroxide in low concentration whereas catalase comes into play when glutathione peroxidase is saturated with the substrate (Gaetam et al., 1989). However, no significant change in SOD levels was demonstrated.

Table 3: Effect of Diosgenin and BHA on Free Radical Scavenging Activities in the Hepatic System of Mice

Group	Treatment (14 Days)	GSH GR (N Moleg Tissue) Consumed/Protein)	GSH (N Moles of NADPH min/mg	GPx (N Moles of NADPH Consumed/ min/mg Protein)	SOD (Specific Activity Expressed as µ Mole/mg Protein)	CAT(µ Mole H_2O_2 Consumed/ Min/mg protein)
I(n = 10)	Negative Control(vehicle)	1.88±0.74	19.24±0.74	15.36±1.22	8.63 ±0.86	104.16 ±5.61
II(n = 6)	Diosgenin (30mg/kg b. w.)	2.27±0.03[a]	21.33±7.75[a]	18.5±5.66 [a]	8.65 ±1.18	109.15±0.71[b]
III(n = 6)	Diosgenin (50mg/kg b. w.)	3.23±0.41[a]	28.67±2.09[a]	24.15±9.65[a]	8.72 ±1.13	118.64±4.73[b]
IV(n = 6)	Positive Control (Normal diet containing 0.75% BHA)	4.87±0.46[a]	28.41±2.59[a]	27.34±3.82[a]	8.75 ±0.88	139.15±5.99[b]

Values are expressed as mean ± S.D of 10 animals. ‘a’ and ‘b’ represent significant changes against negative control at $p < 0.01$ and $p< 0.05$ respectively. Abbreviation: BHA- Butylated hydroxyanisole; GSH – Reduced glutathione; GPx – Glutathione peroxidase; GR – Glutathione reductase; SOD – Superoxide dismutase; CAT– Catalase.

CONCLUSIONS

Our investigation was targeted to identify the possible chemomodulatory potential of diosgenin, a steroidal sapogenin, towards xenobiotic detoxification and free radical scavenging activities. Safety aspect of the modulator was ascertained at selected dose levels based on GOT and GPT levels of treated mice. Oral administration of diosgenin (30mg and 50mg/kg body weight) enhanced the levels of hepatic phase-I and phase-II enzymes that furnish the balance of xenobiotics towards detoxification. Based on these observations, it can be inferred that diosgenin is a bi-functional inducer. Increase in reduced glutathione level, as recorded in our study, act as an important antioxidant to protect against oxidative damage, which has been reported in various studies to promote detoxification of free radicals (Faremi et al., 2008; Singh et al., 2006; Parihar and Hemnani, 2003). The increase in the levels of glutathione reductase, glutathione peroxidase and catalase may be attributed to have biological significance in eliminating reactive free radicals that may have otherwise affect the normal functioning of the cells. The decrease in lipid peroxidation is in correlation with the concomitant increase in free radical scavenging enzymes. We conclude, induction of Phase-II enzymes (GSTs) along with the elevation of free radical scavenging enzymes may protect against cellular damage.

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